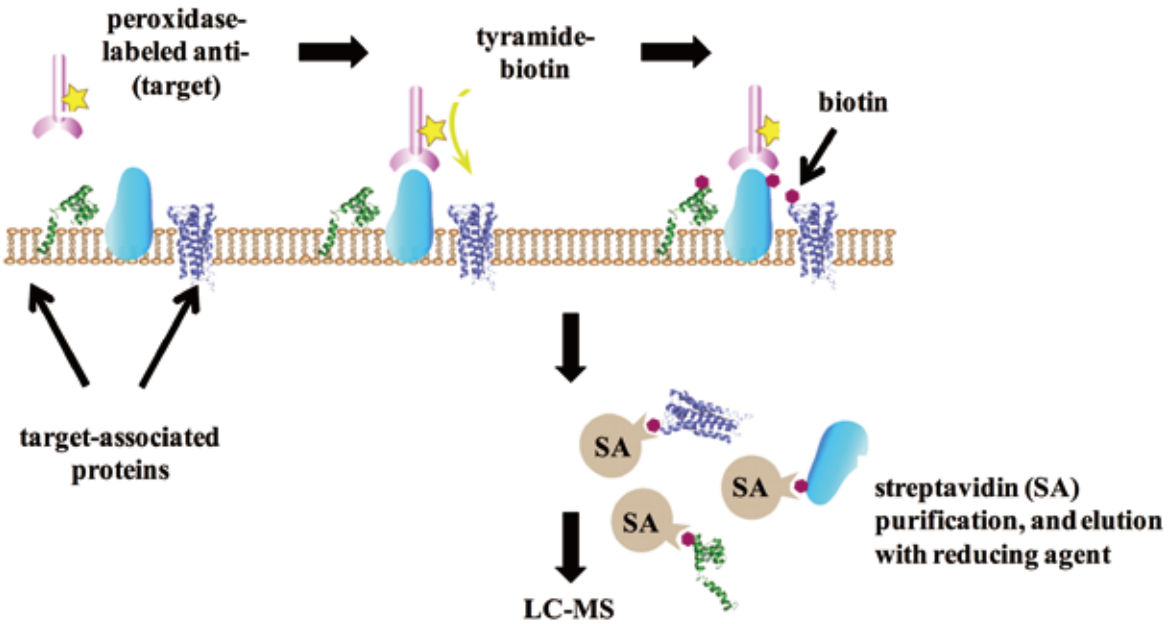
New Technique Developed to Identify Clusters of Proteins on the Plasma Membrane of Immune Cells

When our bodies are under attack from foreign organisms, such as bacteria and viruses, our immune system orchestrates a complex fight-back involving many separate parts. One important component of this response is a type of cell called the B-lymphocyte – it is this cell that is at the forefront of our defence as it identifies and attempts to neutralise invaders.

The B-lymphocyte produces a protein called the B-cell receptor on its surface. The receptor recognises and attaches itself to molecules from the invading organisms, known as antigens. This triggers the B-lymphocyte to divide and to release specialised proteins called antibodies which neutralise the antigens.

There are many aspects of this process that are still not well understood. One reason is because the B-cell receptor does not exist in isolation on the B-lymphocyte surface. Rather, it forms localised clusters together with a number of 'molecular neighbours'. It is these local interactions that control how the lymphocytes divide and replicate and determine the strength of the antibody response. A better understanding of these interactions could ultimately lead to better control of the immune response – for example in vaccine development. However, the molecular contacts within the clusters are relatively weak, and so they are technically difficult to identify.

Now, in an international collaboration, scientists at



Principle of SPPLAT developed in the collaborative research. The antibody-directed targeting of HRP to a surface protein of interest, followed by brief labeling with biotin-tyramide enables proteins in the immediate vicinity of the target to be biotinylated. These are isolated by incubation of the cell lysate with streptavidin-agarose (SA), and elution with reducing agent.

the CAS Institute of Biophysics (IBP), the University of Cambridge's Department of Biochemistry and the Cambridge Centre for Proteomics have developed a technique that allows some of these molecules to be detected. It is published in the 23rd May edition of the *Journal of Biological Chemistry*. The method enables proteins in the immediate vicinity of the B-cell receptor to be chemically tagged in such a way that they can be more easily isolated. The tagged molecules can then be identified using a method called mass spectrometry.

For this initial "proof of principle" experiment, the researchers looked at the B-cell receptor on the surface of a chicken B-lymphocyte and identified molecules that were hitherto not thought to be involved in regulation of the receptor. They show that these molecules combine with the receptor to activate a class of proteins called integrins that are known to play an important role in the response of B-lymphocytes to antigens. Similar molecules occur on the human B-lymphocyte surface, and drugs active against integrins are already used to modulate the immune response. So a long-term implication of this work may be to identify new therapeutic targets for immune regulation.

The work was supervised by Prof. Sarah Perrett from IBP in Beijing, Dr. Tony Jackson from the Department of Biochemistry, Cambridge and Prof. Kathryn Lilley in the Cambridge Centre for Proteomics. The experiments were performed primarily by LI Xuewen, a Ph.D. student in Prof. Perrett's group in Beijing, and Dr. Jo Rees in Cambridge. Assistance in mass spectrometry was also provided by the group of Prof. YANG Fuquan in the IBP Proteomic Core Facility Center, Beijing. The experiments on integrin activation were performed with Prof. Richard Farndale in the Department of Biochemistry, Cambridge.

"In applying this technique, we have addressed a particularly challenging issue," Prof. Perrett remarks: "how do we identify weak and transient, but potentially important, interactions between membrane proteins, which are notoriously difficult to work with?" Her collaborator, Dr. Tony Jackson added: "There are many problems in cell-biology where we would like to identify proteins that group together on the cell surface, and our method could also be applied in these cases. It should therefore be of interest to a wide group of researchers in both the academic and industrial biomedical communities."

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